Application Serial No. 09/656,561

Examiner: S. McCormick

Art Unit: 1661

PATENT M&G: 11378.21US01

followed by thorough cleaning with sterile sea water to remove any traces of antibiotics and fungicide and blotting with sterile filter paper to obtain axenic explants;

- b) culturing the axenic explants on agar plates fortified with PES medium at a temperature ranging between 20-25°C in the presence of cool white fluorescent lights at about 20-50 μ mol photon m⁻² s⁻¹ irradiance and a 12 : 12 light and dark cycle for induction of callus;
- c) excising the callus from the explant after a period of at least 40 days and subculturing the callus on fresh agar plates in the presence of cool white fluorescent lights with 40-60 μ mol photon m⁻² s⁻¹ irradiance and a 12 : 12 light and dark cycle to obtain differentiated densely pigmented oval or spherical shaped micro-propagules;
- d) subculturing thin slices of the pigmented callus in agar plates in Provasoli Enriched Seawater (PES) medium containing plant growth regulators, for a period of about 20 to 40 days, in the presence of cool white fluorescent lights of 20-60 µmol photon m⁻² s⁻¹ irradiance and a 12: 12 light and dark cycle to achieve enhanced somatic embryogenesis and micro-propagule formation in pigmented filamentous callus;
- e) transferring the filamentous calli with somatic embryos to liquid PES medium in an agitated condition for morphogenesis and development of young plantlets with multiple shoots from propagules; and
- f) cultivating algal biomass on a large scale in the sea by growing the young plantlets in enclosed perforated polythene bags.
- 2. (Amended) A method as claimed in claim 1, wherein the material for tissue culture is a Rhodophytic marine algae selected from the group of genera of *Eucheuma*, *Gigartina*, and *Chondrus*.
- 3. (Amended) A method as claimed in claim 1, wherein the material for tissue culture is an algae selected from the group of Eucheuma striatum, Kappaphycus alvarezii, Eucheuma cottonii, Eucheuma denticulatum, Eucheuma spinosum, Eucheuma alvarezii, Eucheuma procrusteanum, Gigartina intermedia, Gigartina exasparata and Chondrus crispus.



Application Serial No. 09/650,561

Examiner: S. McCormick

Art Unit: 1661

PATENT M&G: 11378.21US01

A

4. (Amended) A method as claimed in claim 1, wherein the axenic explants comprise 1 to 6 mm long cuttings with 3-4 mm diameter and are selected from the upper or distal parts of the algae.

A 2

8. (Amended) A method as claimed in claim 1, wherein the calli is subcultured by growing thin slices of pigmented calli as embedded cultures in agar plates containing 0.3-0.6% agar and made in provasoli enriched seawater medium at 20-25°C in the presence of cool white fluorescent light at about 20-50 μ mol photon m⁻² s⁻¹ irradiance with 12:12 light and dark cycle to obtain a profusely branched filamentous pigmented calli in each embedded block.

15. (Amended) A method as claimed in claim 1, wherein the process of formation of somatic embryos through somatic embryogenesis of pigmented callus is further enhanced by adding plant growth regulators including α-naphthalene acetic acid and 6-benzylaminopurine.

 $\int_{\mathcal{U}} 3$

16. (Amended) A method as claimed in claim 1, wherein a harvesting period after at least 60 days can yield a higher biomass than that of a control of parent plants or wherein the biomass yield can be maintained constant and a cultivation period reduced from at least 60 days.

17. (Amended) A method as claimed in claim 1, wherein a two fold increase in growth in fresh weight is achieved over a control of parent plants, without change in carrageenan product yield and gel strength, through micro-propagule formation from pigmented calli.

Please add the following claims.

A4

18. (New) A method as claimed in claim 1, wherein the material for tissue culture is a Phaeophytic marine algae selected from the group of genera of Laminaria, Undaria, Ecklonia, Eisenia, Macrocystis, Sargassum, and Turbinaria.